Investigations on *N*-Nitrosopyrrolidine in Dry-Cured Bacon

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Dry-cured or "country-style" bacon is a low volume specialty product typically made by small producers whose production practices vary widely. These practices include the direct application of dry-cure formulations containing varying concentrations of salt, sugar, flavoring agents, sodium nitrite, and sometimes sodium nitrate, and the use of lengthy curing and processing times. Because of the possibility of generating higher levels of *N*-nitrosopyrrolidine (NPYR) after frying in this product type compared with pump-cured bacon, an investigation was carried out on dry-cured bacon obtained from cooperating state or federally inspected establishments. Three different samples from each of the 16 plants were analyzed. Only one sample from each of 2 different producers exceeded the Food Safety and Inspection Service (FSIS) action level of 17 ppb NPYR, indicating that the majority of samples tested were in compliance. A significant correlation ($P < 0.01$) was found between residual $NaNO_2$ prior to frying and NPYR after frying. The elimination of added nitrate in the dry-cure formulations is recommended.

An estimated 15 million pounds of dry-cured or "country-style" bacon is manufactured annually in the United States (J. D. Kemp, University of Kentucky, personal communication, 1987). This specialty product, which is usually associated with the southeastern part of the United States, is typically made by small producers whose processing practices vary widely. These practices include the use of cure formulations that contain varying concentrations of NaCl, sugar, and $NaNO_2$ sometimes in combination with $NaNO_3$, as well as different flavoring agents. These components are applied directly to the pork bellies in a dry form; then the bellies are held to permit diffusion of the salts throughout the product prior to smokehouse treatment. The heterogeneous nature of the cure application may cause localized areas of the product to have a high nitrite content. The more lengthy curing time for dry-cured bacon compared with that for brine-pumped bacon may produce more amine precursor through microbial-enzymatic action, thereby creating the potential for greater formation of the carcinogen *N*-nitrosopyrrolidine (NPYR).

Pensabene et al. found from 39 to 89 ppb NPYR in fried dry-cured bacon in the 7 commercial samples that they tested (1). A higher incidence and concentration of NPYR (trace to 320 ppb) was found in dry-cured bacon from 15 different producers compared with levels in other dry-cured products that included ham, picnics, shoulders, and dried beef (2). In 1981, A Food Safety and Inspection Service (FSIS) report indicated a 15% incidence of fried dry-cured bacon that exceeded the 17 ppb action level ("Dry-Cured Bacon Survey Report," U.S. Dept of Agriculture, FSIS internal report, unpublished, 1981). Although this was lower than the incidence found in earlier, more limited studies ("Study to Survey Nitrosamine Levels in Dry-Cured Bacon, Hams and Shoulders," U.S. Dept of Agriculture, FSIS internal report, unpublished, 1980), dry-cured bacon was still considered a potential problem. All of these findings identified dry-cured bacon as the one product type that should warrant further investigation. For this reason, we carried out a survey of dry-cured bacon obtained from a number of cooperating state and federally inspected establishments where the ingredient composition, conditions, and other processing details were available. The objective was to use the results to identify those processing parameters and practices that contributed to high NPYR formation and to make recommendations to processors that would enable them to reduce the nitrosamine levels should it become necessary.

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Experimental

Caution: *N*-Nitrosamines are potential carcinogens. Exercise care in handling these compounds.

Reagents and Apparatus

(a) *Dry-cured bacon.*—Dry-cured bacon from 3 different bellies was obtained from 16 processors (48 samples) immediately after production, and samples were shipped to ERRC within 1 day.

(b) *N-Nitrosoazetidine (NAZET) internal standard.*—0.10 μg NAZET/mL dichloromethane (DCM).

(c) *N-Nitrosohexamethyleneimine (NHMI) internal standard.*—0.10 μg NHMI/mL DCM.

(d) *N-Nitrosopyrrolidine (NPYR) standard solution.*—0.10 μg NPYR and 0.10 μg NAZET/mL DCM or 0.10 μg NPYR and 0.10 μg NHMI/mL DCM. NPYR, NAZET, and NHMI were synthesized from their corresponding amines and sodium nitrite following the general method described previously (3).

(e) *Gas chromatograph–thermal energy analyzer (GC-TEA).*—Operating conditions: 2.7 m × 3.2 mm stainless steel column packed with 15% Carbowax 20 M-TPA on 60–80 mesh Gas-Chrom P; He carrier gas 35 mL/min; injector 200°C; TEA furnace 450°C; TEA vacuum 1.5 mm; liquid nitrogen cold trap; column 180°C isothermal for fried bacon extracts, 190°C isothermal for bacon-dripping extracts.

(f) *Other reagents and apparatus.*—As previously described (4–10).

Procedures

(a) *Bacon frying.*—Rind-free bacon (⅛ in./slice) was fried in preheated Farberware electric frying pan for 4.5 min (2.25 min/side) at calibrated temperature of 171°C (340°F). Both the fried edible portion and rendered drippings were retained for nitrosamine analysis.

(b) *NPYR in fried bacon.*—Complete details of procedure for analysis of NPYR in fried bacon have been described previously in secs **24.054–24.058** (4). Analyze all samples in duplicate. Briefly, prepare glass column containing acidified Celite. Add to this column ground mixture of fried bacon, anhydrous sodium sulfate, and Celite. Rinse column mixture with pentane–DCM, then elute nitrosamines from column with DCM. Concentrate sample to 1.0 mL and quantitate on GC-TEA system.

(c) *NPYR in bacon drippings.*—Complete details of pro-

Table 1. Nitrite, nitrate, and *N*-nitrosopyrrolidine in dry-cured bacon

Producer	Days in cure	Raw bacon				NPYR, ppb, in cooked bacon			
		$NaNO_2$, ppm		$NaNO_3$, ppm		Fried		Drippings	
		Range	Av.	Range	Av.	Range	Av.	Range	Av.
1	10	22.3–33.6	27.9	42.8–88.2	54.7	3.5–5.1	4.2	18.1–22.8	20.4
2	10	4.2–26.0	12.2	5.9–72.1	27.4	2.6–4.4	3.4	10.9–16.6	13.5
3	3	27.8–49.3	38.8	51.2–77.8	66.5	6.6–14.9	10.5	32.7–43.4	37.2
4	13	10.4–30.7	20.2	34.2–68.5	46.5	4.4–8.3	6.8	15.4–28.2	22.4
5	4.5	20.2–39.6	29.5	109.8–200.1	153.6	5.8–8.5	7.0	13.9–21.1	16.7
6	7	102.3–288.7	181.5	55.6–137.2	89.0	7.0–13.1	10.4	36.0–54.5	42.1
7	5	41.0–53.6	45.9	41.4–66.1	53.3	4.9–5.8	5.3	20.0–23.7	21.3
8	4	21.6–37.2	28.5	0.0–100.5	28.7	5.3–9.3	7.8	6.5–9.7	7.6
9	6	12.2–19.8	15.4	54.8–104.9	83.3	4.5–6.2	5.5	13.5–20.5	16.8
10	7	15.0–29.7	20.9	0.4–31.7	14.5	6.7–18.0	11.3	21.0–33.7	26.4
11	7	22.0–36.2	26.6	30.6–55.1	39.4	4.4–6.8	5.4	18.3–35.5	28.3
12	4	13.6–39.3	28.0	7.6–65.8	31.8	5.5–13.4	8.6	19.4–36.7	26.0
13	12	44.9–50.8	47.9	42.0–87.2	60.1	12.6–22.3	15.9	17.4–26.3	22.5
14[a,b]	56	2.2–5.8	3.4	370.2–766.8	525.9	4.8–10.1	6.6	17.0–28.9	22.1
15	33	11.3–73.8	33.1	36.8–144.7	74.7	9.9–11.9	10.7	24.3–40.2	32.9
16	14	9.4–42.0	21.6	5.2–44.6	28.9	5.3–8.1	6.4	18.0–26.2	22.8

[a] Pork side meat.
[b] Also contained *N*-nitrosopiperidine, 3.3–6.1 ppb.

cedure for analysis of NPYR in bacon drippings have been described previously (5). Briefly, steam-distill drippings from 5N sodium hydroxide. Extract aqueous distillate with DCM, then wash DCM extract with acid and base to remove interfering compounds. Concentrate sample to 1.0 mL and quantitate on GC-TEA system.

(d) *Sodium nitrite–nitrate.*—Determine values on 10.0 g uncooked sample-sodium nitrite by modified Griess-Saltzman procedure (6); sodium nitrate was determined by cadmium reduction method (7).

(e) *Fat, moisture, protein.*—The following AOAC methods (4) were used: fat by Soxhlet extraction, sec. **24.005**; moisture by oven drying method, sec. **24.003**; protein by Kjeldahl procedure, secs **24.038-24.040**.

(f) *pH.*—pH was determined on 10.0 g uncooked sample as in secs **28.025-28.026**.

(g) *Peroxide value.*—Peroxide value (PV) was determined on 20.0 g uncooked sample by using modification of procedure for determining PV in fats and oils, secs **28.025-28.026** (8). Briefly, homogenize sample for 2 min with 10 g anhydrous sodium sulfate and exactly 100 mL $CHCl_3$ in a 250 mL Virtis flask. Filter homogenate through Whatman No. 2 paper containing 10 g anhydrous Na_2SO_4. Pipet 25 mL aliquot into 250 mL flask, add 30 mL acetic acid and 2 mL KI solution, and place flask in dark for exactly 2 min. After 2 min, add 50 mL water and 2 mL starch solution, and titrate to clear end point with sodium thiosulfate.

(h) *Sodium chloride.*—Salt value was determined on 10.0 g uncooked sample following modification of Mohr method for chloride (9). Briefly, homogenize sample for 5 min with 50 mL hot water in 250 mL Virtis flask. Using 50 mL hot water, transfer sample to 250 mL beaker and heat on steam bath for 30 min. Filter hot sample through glass wool using another 50 mL hot water into 250 mL Erlenmeyer flask. Add potassium chromate and titrate.

(i) *Water activity.*—Water activity was determined using Rotronic Hygroskop DT instrument.

Statistical Analysis

The General Linear Models (GLM) procedure of Statistical Analysis System PC software distributed by SAS Institute, Inc., was used to interpret results according to methods of Snedecor and Cochran (10).

Results and Discussion

Because dry-cured bacon has a lower water activity, a_w, than pump-cured bacon, there is less water to evaporate during frying. The temperature-dependent reaction would be more rapidly attained in the dry-cured product, which could result in higher NPYR values under the same cooking conditions. The details of the experiments leading to the reference standard of cooking for dry-cured bacon, taking into account such factors as degrees of doneness, slice thickness, frying time and temperature, yields, proximate analysis, and water activity, are described in the FSIS internal report, "Dry-Cured Bacon Survey," 1981. Thus, the FSIS frying protocol for this product of 340°F for 4.5 min (2.25 min/side) employing ⅛ in. slices was used for the present study instead of 370°F for 6 min normally used for pump-cured bacon. To help evaluate reproducibility of the NPYR values and to determine if they were representative, the analyses were performed in duplicate on 3 different dry-cured bacon samples from each establishment. The solid phase extraction method used for the determination of NPYR was compared previously against 2 other methods for this type of product and was shown not to produce artifactual nitrosamines (11).

The range and mean of residual $NaNO_2$ and of $NaNO_3$ in the uncooked and NPYR in the fried bacon and its cooked-out drippings from each establishment are shown in Table 1. The overall range for NPYR in fried bacon was 2.6–22.3 ppb and the mean was 7.9 ppb. Analysis of the data showed a highly significant ($P < 0.01$) correlation between residual $NaNO_2$ in uncooked bacon and NPYR in the edible fried portion and cooked out drippings. This finding is in agreement with that first found by Pensabene et al. with respect to fried pump-cured bacon (12). Only single samples from 2 different producers (10, 13) exceeded the FSIS action level of 17 ppb NPYR. One producer's bacon (6) contained 102.3–288.7 ppm residual $NaNO_2$ compared with an overall average of 36.1 ppm and had a higher NaCl content than the other samples tested. Only 7.0–13.1 ppb NPYR was found in the fried bacon from this producer despite the indication that the incorrect amount of cure/amount meat was used.

The overall NPYR results from the corresponding drippings ranged from 6.5 to 54.5 ppb, with a mean of 23.6 ppb. This is consistent with results reported by Sen (13) who found the concentration of volatile nitrosamines in cooked-out ba-

con fat to be more than twice that present in cooked bacon. This is largely because the NPYR precursor(s) are located almost exclusively in the adipose, not lean, tissue (14, 15). The total NPYR yield from adipose tissue, including NPYR released (volatilized) during cooking, is at least 12 times in excess of that derived from lean tissue (16). There is also some indication that the nitrosating species is not N_2O_3 generated directly from nitrite, but generated indirectly through a lipid-NO_x reaction product (17). Analysis of variance (ANOVA) of the data showed that the repeatability for NPYR was 0.48 ppb in the edible fried bacon and 1.12 ppb NPYR in the drippings.

Interestingly, bacon from company 12 which had the typical oxidized fat-like off odor associated with dry-cured meats averaged only 8.6 ppb NPYR. Samples from company 14 were actually a pepper-coated, dry-cured product-pork side meat. The fried product and drippings contained an average of 6.6 and 22.1 ppb NPYR, respectively. All 3 samples also contained 3.3–6.1 ppb *N*-nitrosopiperidine (NPIP) in the drippings despite the low residual nitrite (2.2–5.8 ppm) available at the time of frying. NPIP has been detected in cured products processed with a cure premix containing both nitrite and black pepper that had reacted to form the nitrosamine prior to its addition to the meat (18). Because the pork side meat was a single sample, we purchased 3 samples of the same product of one company to obtain additional data. They contained 15.7, 8.8, and 20.9 ppm residual $NaNO_2$ and gave 29.1, 1.5, and 21.5 ppb NPYR in the edible portion, and 47.0–49.6 ppb in the drippings with 2.4 ppb NPIP in the latter sample. The second sample yielded insufficient drippings for analysis. Given the fact that 2 of the 3 samples had the highest NPYR values encountered in any of the dry-cured bacon and that this product type is used exclusively for cooking and flavoring purposes, additional investigation is warranted.

Peroxide value (PV) was determined in at least one of the 3 samples from each producer to assess the degree of oxidized fat present. The values ranged from 0.50 to 1.90 except for pork side meat, where values for those 3 samples ranged from 5.65 to 6.25. No significant correlations were found between PV values and NPYR in either the fried bacon or its drippings.

No significant correlations were found between NPYR (edible portion and drippings) and days in cure of the product. Excluding the pork side meat from company 14, whose curing time was 56 days, the conventional dry-cured bacon curing time varied widely from 3 to 33 days with a mean of 9 days. It is interesting that 6 of the processors used a curing time of less than 7 days. The curing time previously used for making traditional dry-cured bacon was 2 days per pound of belly, which typically weigh 15–20 lb (19). It appears that most of the current producers of dry-cured bacon have successfully employed shorter curing times. This may, in part, help account for the recent relatively low NPYR values because there is less opportunity for the bacterial/enzymatic degradation of meat components to form the nitrosamine precursor(s). It would also help explain the wide range among the water activities (a_w), from 0.90 to 0.99 with a mean value of 0.94. The a_w results show a wide variation between processors and between samples from the same producer. As expected, there was a significant correlation ($P < 0.05$) between a_w and NaCl content. The range of 1.05–5.08% NaCl and mean of 2.34% suggests that processors are using less salt than previously used for this product type.

One producer (No. 8) with the lowest NPYR in the drippings (average 7.6 ppb), but not in the fried bacon (average 7.8 ppb) used the reductant sodium ascorbate, which has been shown to be effective in reducing NPYR in pump-cured bacon. The dry-cure premix also contained sodium carbonate as a buffering agent to prevent the rapid destruction of nitrite prior to use. Sodium carbonate would tend to raise the pH of the product slightly (pH 6.6) so that presumably less nitrosating species N_2O_3 from nitrous acid was available at the time of frying. Company 10 who also used a commercial premix containing nitrite and sodium carbonate, but no ascorbate, had samples (pH 6.3) with much higher levels of NPYR in fried bacon and its drippings than those from company 8. When the values for pork side meat are eliminated, pH values for the rest of the dry-cured bacon ranged from 5.4 to 6.7 with a mean of 6.0. Because of the buffering ability of the meat itself, the use of carbonate buffer, in the amounts used in the premix (1%), had little effect on the overall pH levels of the product. The effect of ascorbate on NPYR formation in company 8 samples was not apparent since none of the other producers used this reductant or its isomer erythorbate. Many of these producers had bacon that contained lower NPYR. Ascorbate has not been used extensively in dry-cured bacon production. Perhaps this is due to its limited solubility in adipose tissue. Nevertheless, ascorbate/erythorbate should be effective in reducing residual nitrite and, thus, NPYR, if it could be preserved in the premix.

The use of $NaNO_3$ introduces yet another factor insofar as nitrosamine formation is concerned. A number of factors affect the microbial/enzymatic conversion of nitrate to nitrite. These include the composition of bacterial flora and processing and post-processing time/temperature. Considerably within- and between-plant variation would make control of this conversion extremely difficult. In this study, only 3 (companies 5, 9, and 14) of the 16 processors claimed to use $NaNO_3$ in combination with $NaNO_2$ in the cure premix. Taken without the values from company 14, who produced pork side meat, the residual $NaNO_3$ content was generally low, ranging from none detected to 200 ppm with a mean of 57 ppm. Statistical analyses showed no significant correlation between $NaNO_3$ and NPYR levels in either fried dry-cured bacon or its drippings. From 4.8 to 7.8 ppb NPYR was detected in the 2 bacon processors who used $NaNO_3$. This finding, based on a limited number of samples, contrasts with an earlier study in which up to 280 ppb NPYR was found in fried bacon in 12 of 15 samples from 7 of 15 producers who added $NaNO_3$ in the cure (2). This was one of the studies that helped identify dry-cured bacon as the one product type requiring further investigation. The fried bacon from the few producers who employed $NaNO_3$ in this study did not contain higher NPYR than the majority who used $NaNO_2$ alone. Generally short curing times (4–6 days) would help explain the lack of significant nitrate-to-nitrite conversion.

Despite claims by some processors that nitrate is essential to produce "good, high quality" dry-cured bacon and ham, a majority no longer use nitrate. There is overwhelming evidence that nitrite is the source of the nitrosating agent. Nitrite concentrations, higher than those needed for color and flavor development, are needed for *Clostridium botulinum* inhibition. Nitrate is generally considered nonessential in these respects, serving only as an unreliable source of nitrite (20, 21). Recognition of these factors led the Expert Panel on Nitrates, Nitrites and Nitrosamines to recommend that the use of nitrate salts be discontinued in curing all meat and poultry products, except for fermented sausage and dry-cured products (22). In 1978, nitrate was specifically prohibited from use in pump-cured bacon (23).

This study presents evidence for the principal association

between residual $NaNO_2$ prior to and NPYR after frying. Therefore, it is essential to control ingoing and thus residual nitrite. Although somewhat inconsistent with the current finding that no correlation exists between nitrate and NPYR levels, we nevertheless recommend the elimination of nitrate to avoid the *potential* for an additional source of nitrite for nitrosamine formation. There appears to be no need for using nitrate in a dry-cured formulation for making bacon, except if there were compelling quality or safety considerations that are unique to specific producers.

In conclusion, it is recognized that dry-cured bacon is a unique product. Clearly, modern curing practices within the industry have undergone substantial changes; these changes allow better control of the amount of ingoing nitrite through the use of commercial premixes and the elimination of added nitrate.

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